Plant Archives Volume 20 No. 2, 2020 pp. 7560-7566 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF DIFFERENT LEVELS OF *NIGELLA SATIVA* SEEDS (BLACK CUMIN) ON PRODUCTION PERFORMANCE AND SOME IMMUNOLOGICAL PARAMETERS OF BROILER CHICKEN IN AKRE REGION, IRAQ

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Abstract

A total of 240 one day old chicks of commercial hybrid Ross 308 were randomly divided into four treatments and were subdivided into three replicates in each treatment. Seeds of *Nigella sativa* were added to the diet as follows: T1 (control group), no supplemented of *Nigella sativa* Seeds, T2, T3 and T4 contains 1, 2 and 3% of *Nigella sativa* respectively. Birds were weighted at one day old, 7, 14, 21, 28, 35 and 42 days. Feed intake and feed conversion ratio were recorded weekly. At 42 days of age, three birds from each replicate were randomly chosen; slaughtered and lymphoid organs were collected, weighed and expressed as a percentage of carcass weight. Also, 2 ml of blood were collected from three birds of each replicate and analyzed for total cholesterol, uric acid, total protein, glucose, albumin, globulin and triglyceride.

The effect of *Nigella sativa* seeds levels on live body weight showed to be significant in live body weights of chicks at 3, 4, 5 and 6 weeks of age as well on body weight gains of chicks at the 5th and 6th were significant. However, adding 1% of *Nigella sativa* (T2) recorded the highest body weight gain (503.00 g) at the end of the experiment (week-6). The differences in feed intake between chicks having different levels of *Nigella sativa* seeds were significant during the 1st and 2nd weeks, whereas the differences were significant on feed conversion ratio at the 5th and 6th weeks. The differences in the percentages of spleen and bursa due to different levels of *Nigella sativa* seeds were numerical and statistically didn't reveal to the level of significant. The effect of *Nigella sativa* on immunological traits of broiler were found to be significant only on globulin and triglyceride, the highest means of globulin (6.230 g/dl) and triglyceride (93.380 mg/dl) were recorded in T3 (2%) and T4 (3%) respectively.

Key words: Broiler, *Nigella sativa* Seeds, Production Performance and Immunity.

Introduction

Feed additives as antibiotics, prebiotics and probiotics are widely used in poultry diets as growth promoters to improve nutrient utilization and feed efficiency (Hassan and Mandour, 2018). Incorporation of antibiotics as a principal growth promoter in poultry feed often resulted in the incidence of cross resistance among pathogens and also a source of residues in animal body tissues (Schwarz *et al*., 2001).

The black cumin (*Nigella sativa*) is an annual herbaceous plant that belongs to the *Ranunculaceae* family (Al-Gaby, 1998 and Atta, 2003). Black seed (*Nigella sativa L.*) which grows in Asian and Mediterranean countries is also recognized as Black Caraway seed, Habbatul Baraka (the Blessed Seed) and Black Cumin (Abdullah *et al*., 2019). Black seed have several natural properties including anti-parasitic (Mahmoud *et al*., 2002), anti-diabetic effect (Al-Hader *et al*., 2003) and diuretic effect (Zaoui *et al*., 2000). It stimulates the immune system (Al-Beitawi *et al*., 2009) with high nutritional potential and contains 21% protein, 38% carbohydrates and 35% fat (Babayan *et al*., 1978). The main component of Black seed are fixed oils, thymoquinone and nigellone (Abdullah *et al*., 2019); also it include other component such as vitamins C, B, B12, A, niacin, Selenium, magnesium, iron, calicium and potassium (Al-Beitawi *et al*., 2009 and Al-Mufarrej, 2014).

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Table 1: Proximate analysis of *Nigella sativa* seeds.

Nutrient	%
Moisture %	4.40
Dry matter %	95.60
Crud protein %	22.75
Ether extract %	36.25
Ash%	4.45
*Values in the table represent the average of three samples.	

Nigella sativa seeds dietary supplementation has revealed some of its positive effect on broiler chicks' performance (Guler *et al*., 2006; Abu-Dieyeh and Abu-Darwish, 2008), weight gain, feed conversion ratio (AL-Harthi, 2004 and Khan *et al*., 2012), feed intake, internal organ weight percentages, thigh and breast weight percentages (Durrani *et al*., 2007), as well as dressing weight percentage (Halle *et al*., 1999 and Durrani *et al*., 2007). Moreover, the effects of NS feed supplementation on growth performance and carcass measurements of broiler chicks have been explored in some studies.

Several different pharmacological have been traditionally attributed to *Nigella sativa* seeds, honestly as a beaten powder, or as an extract. Purified or as a mixture, metabolites of *Nigella sativa* seeds would current a powerful and therapeutically fascinating exercise on the cardiovascular, respiratory, immune and endocrine systems (Gilani *et al*., 2004 and El-Tahir and Bakeet, 2006). Therefore the objective of this study was to study the effect of feeding different levels of *Nigella sativa* Seeds (Black Cumin) on production performance and some immunological traits of broiler chicks in Akre regions.

Materials and Methods

The experiment conducted using 240 one day old chicks of commercial hybrid Ross 308 at the poultry production farm in Akre region for a period of 42 days. *Nigella sativa* seeds were obtained from a local market of Akre region, the seeds were directly mixed with manually prepared diets in appropriate doses. Birds were randomly distributed into four treatments and three replicates with 20 birds for each replicate. Randomized samples from each formulated rations and *Nigella sativa* seeds were taken, grounded and 0.5 g were used for the chemical determination of nitrogen, ether extract, dry matter and ash using the procedure described by A.O.A.C., (1990) and crud protein was calculated by multiplying N content by 6.25 (Table 1). All diets were formulated to cover the nutreint requirements of N.R.C. (1994). Ingrediants and the compositin of the experimental diets are shown in table 2. First group served as a control (T1), while, the other groups fed diets T2, T3 and T4 contains 1, 2 and 3% of Nigella sativa seeds (black cumin) respectively.

The chicks were fed using two rations: starter (1-21 days) and finisher (22-42 days), with adding *Nigella sativa* seeds levels. The chemical analysis of diet was done in physiological laboratory, in Akre Technical College- Duhok Polytechnic University (Table 2). At one day old, birds from each replicate were weighted and correspondingly measured by a balance at 7, 14, 21, 28, 35 and 42 days, then their body weight gain (BWG) were calculated. Feed intake in each pen (replicate) was recorded and measured weekly and then average daily

Table 2: Ingredients and composition of experimental rations.

Finisher rations				**Starter rations**				**Ingredients**
3.0% (T4)	**2.0% (T3)**	**1.0% (T2)**	**Control (T1)**	**3.0% (T4)**	**2.0% (T3)**	**1.0% (T2)**	**Control (T1)**	
64.35	63.75	65.50	66	59.70	59.25	59.75	60.25	Yellow corn
23.99	24	24.75	25	32.75	32.25	33.0	33.5	Soy Bean meal
1.5	1.5	1.5	2	2	2	2	2	Concentrate
3	2	1	0	2	2	1	0	*Nigella sativa*
4	4	3.5	3.3	1.15	1.25	1	0.5	Sunflower Oil
0.75	1	1	1.45	0.7	1	1	1	Dicalcium phosphate
1	1	1	1.5	0.7	1	1	1.25	Vit. M Premix
1.25	1.5	1.5	1.5	0.75	1	1	1.25	CaCo3
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	NaCl
100	100	100	100	100	100	100	100	Total
Feeding value								
a) calculated								
18.43	18.56	18.52	18.59	22.05	22.03	22.06	22.26	CP%
3173.83	3175.6	3171.21	3176.7	2970.46	2977.06	2978.11	2976.32	ME/kg
b)analyzed								
18.55	18.53	18.57	18.57	22.25	22.3	22.25	22.25	CP%

Table 3: Effect of different levels of *Nigella sativa* seeds on live body weight (g) at different ages of broiler (Mean ± S.E.).

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week
T1	146.250±2.754[a]	307.750±7.000[a]	639.232±12.707[b]	1211.33±13.094[b]	1656.00±37.287[c]	2145.33±30.278[b]
T2	142.000±5.267[a]	368.160±20.452[a]	753.079±8.514[a]	1280.33±24.694[ab]	1757.00± 27.221[b]	2260.00±18.93[a]
T3	142.750±1.421[a]	366.653±19.793[a]	737.259±40.485[ab]	1302.33±42.604[a]	1974.33± 18.977[a]	2237.33±18.747[a]
T4	139.750±4.750[a]	357.495±26.794[a]	753.043±51.365[a]	1322.67±20.218[a]	1955.67± 26.723[a]	2189.00±26.764[ab]
Means with different letters significantly different at p≤0.05.						

feed intake (FI) was calculated. Feed conversion ratio (FCR) calculated as a ratio FI: BWG at the end of each experimental period. At 42 days of age, three birds from each replicate were randomly chosen; slaughtered and lymphoid organs (spleen, bursa of Fabricius) were collected, weighed and expressed as a percentage of carcass weight. Also, 2 ml of blood were collected from the brachial vein from three birds of each replicate (12 birds per treatment) by puncturing wing vein using sterilized syringes and needles. Serum samples were isolated from blood by centrifugation at 2500 rpm for 15 min. After centrifugation, the serum supernatant was carefully separated by a micropipette and preserved in an eppendorf vial at -20^{0}C until determination (Siddiqui *et al*., 2015). Individual serum samples were analyzed for total cholesterol, uric acid, total protein, glucose, albumin, globulin and triglyceride, by using spectrophotometer following the instructions of the commercial lipid profile kit (Crescent Diagnostics).

Statistical analysis was accomplished using the programme of Statistical Analysis System (SAS, 2005). To diagnosing the significant differences between treatments, the proceeding of Duncan's multiple range tests (Duncan, 1955) at level of p≤0.05 was detected.

Results and Discussion

The effect of *Nigella sativa* seeds levels on live body weight showed no significant differences among all treatments at the first 2 weeks of age which could be clarified by the large values of the standard errors due to the large differences among the measurements within replicates. While the differences observed to be significant in live body weights of chickens at 3, 4, 5 and 6 weeks of age. The highest body weights 753.079, 1322.67, 1974.33 and 2260.00 g were recorded for T2, T4, T3 and T2 at weeks 3, 4, 5 and 6 respectively (Table 3). That means the highest live body weight at the end of the experiment was with using T2 which include adding 1% of *Nigella sativa* seeds. These results were in agreement with the findings of Siddiqui *et al*., (2015) who found that using 3% dose of N. sativa seed supplementation in the diet significantly improved the average body weight compared with the control treatments. While Mohamed *et al*., (2010) reported that the body weight of the protected group plus *Nigella sativa* was non-significantly increased compared to the dimethylaminoazobenzene treated group. The positive effect of supplementation with 1% black cumin seed on performance may be due to its antimicrobial effects on the pathogenic bacteria, fungi and parasites in the digestive system (Gilani *et al*., 2004).

Table 4 showed that the differences in body weight gains of chicks at the 1st, 2nd, 3rd and 4th week due to using different levels of *Nigella sativa* seeds were not significant, while the differences in the 5th and 6th were significant. However, adding 1% of *Nigella sativa* (T2) recorded the highest body weight gain (503.00 g) at the end of the experiment (week-6). This may be due to the component of *Nigella sativa* which increase the immunity

Table 4: Effect of different levels of *Nigella sativa* seeds on body weight gain (g) at different ages of broiler (Mean ± S.E.).

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week
T1	106.917±3.321[a]	161.500±5.008[a]	331.482±6.120[a]	572.101±24.600[a]	444.667±24.251[b]	489.33±27.630[a]
T2	102.00±5.268a	226.16±24.558[a]	384.919±20.901[a]	527.255±33.174[a]	476.667±15.191[b]	503.00±45.764[a]
T3	102.750±1.421[a]	223.903±18.375[a]	370.605±23.206[a]	565.075±28.793[a]	672.00±23.629[a]	263.00±28.442[b]
T4	100.417±4.856[a]	217.745±31.213[a]	395.548±24.835[a]	569.623±41.847[a]	633.00±40.857[a]	233.33±21.851[b]
Means with different letters significantly different at p≤0.05.						

Table 5: Effect of feeding different levels of *Nigella sativa* seeds on feed intake (g) at different ages of broiler (Mean ± S.E.).

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week
T1	127.671±4.009[ab]	253.829±13.016[b]	422.373±8.171[a]	648.601±16.053[a]	730.368±10.915[a]	871.500±19.172[a]
T2	137.974±1.273[a]	292.982±1.522[a]	446.00±9.489[a]	693.789±15.449[a]	737.237±18.926[a]	912.482±55.811[a]
T3	124.754±1.042[b]	267.855±18.648[ab]	431.956±16.506[a]	656.202±22.658[a]	720.829±19.408[a]	954.482±37.082[a]
T4	129.833±5.085[ab]	278.500±4.645[ab]	423.700±9.892[a]	630.050±25.931[a]	756.667±36.033[a]	899.333±44.598[a]
Means with different letters significantly different at p≤0.05.						

Table 6: Effect of feeding different levels of *Nigella sativa* seeds on feed conversion ratio at different ages of broiler (Mean ± S.E.).

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week
T1	1.196±0.046[a]	1.578±0.119[a]	1.274±0.003[a]	1.139±0.063[a]	1.653±0.100[b]	1.788±0.064[a]
T2	1.359±0.061[a]	1.327±0.146[a]	1.170±0.086[a]	1.324±0.075[a]	1.552±0.084[b]	1.825±0.072[a]
T3	1.215±0.023[a]	1.222±0.174[a]	1.173±0.079[a]	1.203±0.149[a]	1.074±0.025[a]	3.734±0.513[b]
T4	1.303±0.106[a]	1.337±0.203[a]	1.077±0.049[a]	1.112±0.044[a]	1.210±0.126[a]	3.900±0.305[b]
Means with different letters significantly different at p≤0.05.						

and accordingly increase the body weight gain. The mentioned results were fully disagreed with the previous study by Çetin *et al*., (2008). Abel-Mageed, (2002) reported that substitution of soybean meal by 3% *Nigella sativa* meal in broiler diets significantly increased body weight gain at 42 days of age. Moreover, the body weight gains improved by black seed extract supplemented diet due to the presence of fat soluble unidentified factors and Vit. F group (a mixture of essential fatty acids including linoleic, linolenic and arachidonic acid) in supplemented herbal feed additives, which have been essential for growth (Murray *et al*., 1993). The results obtained by Nadia (2003) showed that the addition of 1, 2 or 3 % of black seed either during the finishing period (29-49 days of age) or the total period (7-49 days of age) of growth, improved the broiler appetite significantly where the chicks consumed more feed than control group. Miraghaee *et al*., (2011) showed that supplementing 1% *Nigella sativa* improved body weight gain of broilers at starter and grower periods (P≤0.05). While, Siddiqui *et al*., (2015) noticed that body weight gains were improved by using 3% dose of *N. sativa* seed supplementation in the diet.

The differences in feed intake between chicks having different levels of *Nigella sativa* seeds were numerical but not significant during the weeks 3-6, whereas the differences in the 1st and 2nd weeks were significant. The chicks having T2 (1%) of *Nigella sativa* recorded significantly the highest feed intake in the first 2 weeks (Table 5). Such differences in feed intake could be due to the content of Black cumin seeds used in traditional medicine as diuretic and antihypertensive (Zaoui *et al*., 2000); digestive and appetite stimulant (Gilani *et al*., 2004); antidiarrheal (Gilani *et al*., 2001); analgesic (Khanna *et al*., 1993; Khan *et al*., 1999); anthelmintic (Agarwal *et al*., 1979; Chowdhury *et al*., 1998) and antibacterial agents (Ferdous *et al*., 1992; El-Kamali *et al*., 1998). Additionally, other studies showed that black cumin assumed to be antidiabetic (Meral *et al*., 2001); anticancer (Abuharfeil *et al*., 2001; Farah and Begum, 2003); anti-inflammatory (Al-Ghamdi, 2001); spasmolytic and bronchodilatory (Gilani *et al*., 2001); hepatoprotective (Janbaz *et al*., 2003); renal protective (Badary *et al*., 2000) and possessing antioxidant properties (Mansour *et al*., 2002). That means the seeds are very rich and diverse in chemical composition. They contain amino acids, proteins, carbohydrates, fixed and volatile oils (Rajsekhar and Kuldeep, 2011). Çetin *et al*., (2008) reported that feed consumption or intake reduced linearly by increasing doses of black seed extract in 0 to 12 weeks of age. Also, Siddiqui *et al*., (2015) noticed that the feed intake decreased by 3.0% seed or 0.4% acetone extract of *N. sativa* supplemented diets compared with the control treatments. Al-Mufarrej, (2014) showed that chicks fed 2.5% uncrushed *Nigella sativa* seeds had higher (P≤0.05) cumulative feed consumption compared with the other dietary treatments (control, 1.5%, 2.0%, 2.5%, 3.0% crushed *Nigella sativa* seeds and 1.5%, 2.0% and 3.0% uncrushed *Nigella sativa* seeds).

Using different levels of *Nigella sativa* seeds didn't affect the feed conversion ratio (FCR) at the 1st to the 4th weeks of age, while its effect was significant on the trait at 5th and 6th weeks. The best FCR (1.788 g/g) at the last week of the experiment (week-6), recorded for T1 (control group) where the *Nigella sativa* seeds didn't added to the diet (Table 6). These results suggested that improvement in FCR could be related to the better feathering of chickens fed with a diet supplemented with *Nigella sativa* seeds. Miraghaee *et al*., (2011) showed that supplementing 1% *Nigella sativa* improved FCR of broilers at starter and grower periods (P≤0.05). Also, Al-Mufarrej, (2014) noticed that chicks fed 1.5% crushed *Nigella sativa* seeds improved FCR (P≤0.05) compared with the control group or other dietary treatments. Siddiqui *et al*., (2015) claimed that FCR decreased by 3% seed or 0.4% acetone extract of N. sativa supplemented diets compared with the control treatments. The increase feed consumption of quail fed 4.0 or 8.0% *Nigella sativa* seed

Table 7: Effect of feeding different levels of *Nigella sativa* seeds on lymphoid organs of broiler at 42 days of ages (Mean ± S.E.).

Treatments	Traits	
	Spleen%	Bursa %
T1	0.122±0.035[a]	0.134±0.035[a]
T2	0.154±0.008[a]	0.135±0.038[a]
T3	0.126±0.019[a]	0.160±0.048[a]
T4	0.142±0.021[a]	0.221±0.008[a]
Means with different letters significantly different at p≤0.05.		

Table 8: Effect of feeding different levels of *Nigella sativa* seeds on immunological traits of broiler at 42 days of age (Mean ± S.E.).

Treat-ment	Traits						
	Glucose mg/dl	Albumin g/dl	Globulin g/dl	Cholesterol mg/dl	Total protein g/dl	Triglyceride mg/dl	Uric acid mg/dl
T1	242.00±7.023[a]	1.820±0.038[a]	5.556±1.129[b]	70.00±11.00[a]	3.737±0.093[a]	55.930±4.266[b]	14.467±0.088[a]
T2	233.66±0.666[a]	1.910±0.010[a]	5.793±0.157[ab]	89.00±11.93[a]	3.883±0.154[a]	81.980±4.826[ab]	14.600±0.346[a]
T3	227.33±14.169[a]	1.916±0.024[a]	6.230±0.263[a]	85.00±5.507[a]	4.313±0.239[a]	63.473±12.705[b]	13.233±1.128[a]
T4	249.00±4.509[a]	1.913±0.044[a]	5.820±0.189[ab]	88.66±3.756[a]	7.240±3.465[a]	93.380±9.193[a]	14.500±0.651[a]
Means with different letters significantly different at $p \leq 0.05$.							

due to improve the quail appetite. But the increase level up to 16 % resulted decrease of feed consumption. Regarding with feed conversion ratio the average ranged between 2.93 to 3.24 g feed/g gains. The obtained results showed that insignificant differences were observed for FCR among dietary experimental groups.

The differences in the percentages of spleen and bursa due to different levels of *Nigella sativa* seeds were numerical and statistically didn't reveal to the level of significant (Table 7). However increasing the level of *Nigella* seeds increased the weight of spleen and bursa and accordingly increase the spleen% and bursa% at 42 days of age due to improving the immune system. Abaza, (2001) indicated that insignificant differences were observed among groups of broiler fed diets containing different levels of black seeds for spleen and gizzard weight. Moreover, Alimohamadi *et al*., (2014) claimed that the weights of thymus and bursa of Fabricius in the birds fed diets (8gm/kg black seed), (8gm/kg cumin seeds) or probiotic were higher than those of the birds fed the control diet ($p \leq 0.05$).

The effect of *Nigella sativa* on immunological traits of broiler were found to be significant only on globulin and triglyceride, the highest means of globulin (6.230 g/dl) and triglyceride (93.380 mg/dl) were recorded in T3 (2%) and T4 (3%) respectively. Whereas the differences in other traits of broiler including glucose, albumin, cholesterol, total protein and uric acid due to using different levels of *Nigella sativa* were not significant (Table 8). However the highest means of the mentioned traits were 249.00 mg/dl, 1.916 g/dl, 89.00 mg/dl, 7.240 g/dl and 14.600 mg/dl with using T4, T3, T2, T4 and T2 respectively. The normal globulin values indicate good immunity status of the heat stressed chicks and the slight increase in globulins may be due to the immuno-stimulant effect of NS (Aqel, 1993). Toghyani *et al*., (2010) reported no significant effects of black seed and peppermint on serum lipids. Miraghaee *et al*., (2011) studied the effect of different forms and levels of N.S. on blood plasma constituents of heat stressed broiler chicks and claimed that plasma total proteins, albumin (A), globulin (G) and A/G ratios were slightly affected by feeding treated diets compared to the control, but differences failed to be significant. Whereas, control group showed the highest level of total lipids (0.71g/dl), however, no significant differences were detected among treatments. Results of Alimohamadi *et al*., (2014) showed that supplementation of *N. sativa* decreased ($P \leq 0.05$) serum total cholesterol and triglycerides levels while albumin levels increased significantly, while, no significant effects were noticed in serum glucose, globulin. Siddiqui and Sayed, (2015) noticed that the effects of black seed extracts supplemented diet on serum metabolites at 20 and 40 days on total cholesterol, HDL cholesterol and triglyceride of commercial broiler chicks in different dietary treatments during experimental periods were significant. Siddiqui *et al*., (2015) stated that serum triglycerides content didn't affect by feeding either *Nigella sativa* seed powder or acetone extracts supplemented until 3-weeks. Whereas cholesterol contents in blood serum remained statistically unchanged by dietary supplementation of seed powder up to 2-weeks and then significantly decreased comparing with those of control.

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